

Claims

1. A method for identifying a nucleic acid that modulates the function of a vertebrate cell, said method comprising the steps of:

(a) transforming a population of vertebrate cells with a double stranded RNA expression library, wherein at least two cells of said population of cells are each transformed with a different nucleic acid from said double stranded RNA expression library, wherein said transformed nucleic acid is capable of forming double stranded RNA, and wherein said transformation and said formation of double stranded RNA are carried out under conditions that inhibit or prevent an interferon response or a double stranded RNA stress response;

(b) selecting a vertebrate cell in which said nucleic acid is expressed in said cell; and

(c) assaying for a modulation in the function of said cell, wherein said modulation identifies a nucleic acid that modulates the function of said vertebrate cell.

2. A method for identifying a nucleic acid that modulates expression of a target nucleic acid in a vertebrate cell, said method comprising the steps of:

(a) transforming a population of vertebrate cells with a double stranded RNA expression library, wherein at least two cells of said population of cells are each transformed with a different nucleic acid from said double stranded RNA expression library, wherein said transformed nucleic acid is capable of forming double stranded RNA, and wherein said transformation and said formation of

double stranded RNA are carried out under conditions that inhibit or prevent an interferon response or a double stranded RNA stress response;

(b) selecting a vertebrate cell in which said nucleic acid is expressed in said cell; and

5 (c) assaying for a modulation in the expression of a target nucleic acid in said cell, wherein said modulation identifies a nucleic acid that modulates expression of said target nucleic acid.

10 3. A method for identifying a nucleic acid that modulates the biological activity of a target polypeptide in a vertebrate cell, said method comprising the steps of:

(a) transforming a population of vertebrate cells with a double stranded RNA expression library, wherein at least two cells of said population of cells are each transformed with a different nucleic acid from said double stranded RNA expression library, wherein said transformed nucleic acid is capable of forming double stranded RNA, and wherein said transformation and said formation of double stranded RNA are carried out under conditions that inhibit or prevent an interferon response or a double stranded RNA stress response;

15 (b) selecting for a vertebrate cell in which said nucleic acid is expressed in said cell; and

20 (c) assaying for a modulation in the biological activity of a target polypeptide in said cell, wherein said modulation identifies a nucleic acid that modulates the biological activity of said target polypeptide.

4. The method of claim 1, wherein at most one nucleic acid is stably integrated into a chromosome of each cell.

5 5. The method of claim 1, said method further comprising:
(d) identifying said nucleic acid by amplifying said nucleic acid and sequencing said amplified nucleic acid.

10 6. The method of claim 1, wherein said double stranded RNA expression library comprises cDNAs derived from said cells.

7. The method of claim 1, wherein said double stranded RNA expression library comprises randomized nucleic acids.

15 8. The method of claim 1, wherein said double stranded RNA expression library is a nuclear double stranded RNA expression library.

9. The method of claim 1, wherein said double stranded RNA expression library is a cytoplasmic double stranded RNA expression library.

20 10. The method of claim 1, wherein said cell is a mammalian cell.

11. The method of claim 10, wherein said cell is a human cell.

12. The method of claim 10, wherein said cell is selected from the group consisting of a cancer cell, a cell of the immune system, a stem cell, a neuronal
5 cell, a muscle cell, and an adipocyte.

13. The method of claim 1, wherein said nucleic acid is contained in a vector.

10 14. The method of claim 13, wherein said vector comprises an RNA polymerase II promoter, an RNA polymerase I promoter, an RNA polymerase III promoter, or a mitochondrial promoter.

15 15. The method of claim 13, wherein the sense strand and the anti-sense strand of said double stranded RNA are transcribed from the same nucleic acid using two convergent promoters.

20 16. The method of claim 13, wherein said nucleic acid comprises an inverted repeat such that upon transcription said nucleic acid forms a double stranded RNA.

17. The method of claim 1, wherein said assaying comprises measuring an

event selected from the group consisting of cell motility, apoptosis, cell growth, cell invasion, vascularization, cell cycle events, cell differentiation, cell dedifferentiation, neuronal cell regeneration, and the ability of a cell to support viral replication.

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18. The method of claim 1, wherein said double stranded RNA is between 5 and 100 nucleotides in length, inclusive.

10 19. The method of claim 1, wherein said double stranded RNA is at least 100 nucleotides in length.

20. The method of claim 19, wherein said double stranded RNA is at least 250 nucleotides in length.

15 21. The method of claim 20, wherein said double stranded RNA is at least 500 nucleotides in length.

22. The method of claim 21, wherein said double stranded RNA is at least 1000 nucleotides in length.

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23. A method for identifying a nucleic acid that modulates the function of a vertebrate cell, said method comprising the steps of:

(a) transforming a population of vertebrate cells with a double stranded RNA under conditions that inhibit or prevent an interferon response or a double stranded RNA stress response;

5 (b) selecting a vertebrate cell in which said double stranded RNA is expressed; and

(c) assaying for a modulation in the function of said cell, wherein said modulation identifies a nucleic acid that modulates the function of said cell.

10 24. A method for identifying a nucleic acid that modulates expression of a target nucleic acid in a vertebrate cell, said method comprising the steps of:

(a) transforming a population of vertebrate cells with a double stranded RNA under conditions that inhibit or prevent an interferon response or a double stranded RNA stress response;

15 (b) selecting a vertebrate cell in which said double stranded RNA is expressed; and

(c) assaying for a modulation in the expression of a target nucleic acid in said cell, wherein said modulation identifies a nucleic acid that modulates expression of said target nucleic acid.

20 25. A method for identifying a nucleic acid that modulates the biological activity of a target polypeptide in a vertebrate cell, said method comprising the steps of:

(a) transforming a population of vertebrate cells with a double stranded

RNA under conditions that inhibit or prevent an interferon response or a double stranded RNA stress response;

(b) selecting a vertebrate cell in which said double stranded RNA is expressed; and

5 (c) assaying for a modulation in the biological activity of a target polypeptide in said cell, wherein said modulation identifies a nucleic acid that modulates the biological activity of said target polypeptide.

26. The method of claim 23, said method further comprising:

10 (d) identifying said nucleic acid by amplifying said nucleic acid and sequencing said amplified nucleic acid.

27. The method of claim 23, wherein said double stranded RNA is derived from said cells.

15 28. The method of claim 23, wherein said double stranded RNA is between 5 and 100 nucleotides in length, inclusive.

29. The method of claim 23, wherein said double stranded RNA is at least
20 100 nucleotides in length.

30. The method of claim 29, wherein said double stranded RNA is at least

250 nucleotides in length.

31. The method of claim 30, wherein said double stranded RNA is at least 500 nucleotides in length.

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32. The method of claim 31, wherein said double stranded RNA is at least 1000 nucleotides in length.

33. The method of claim 23, wherein each cell is transformed with at most

10 one double stranded RNA.

34. The method of claim 23, wherein said cell is a mammalian cell.

35. The method of claim 34, wherein said cell is a human cell.

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36. The method of claim 23, wherein said population is transformed with at least 100 different double stranded RNAs.